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CHEMICAL BIOLOGICAL CENTER

U.S. ARMY SOLDIER AND BIOLOGICAL CHEMICAL COMMAND

ECBC-TR-067

ACUTE INHALATION TOXICITY OF CHEMICALLY NEUTRALIZED HD IN RATS

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Aberdeen Proving Ground, MD 21010-5424

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13. ABSTRACT *(Maximum 200 words)*

The chemical agent HD (sulfur mustard) was neutralized by hot water (90 °C) hydrolysis to produce a markedly less toxic wastestream (product solution). An acute inhalation toxicity test was conducted on the HD neutralized solution to assess the inhalation hazard per Department of Transportation (DOT) guidelines. Sprague-Dawley rats were exposed for 1 hr to an aerosol concentration of 5.4 mg/L, which consisted primarily of the HD hydrolysis solution of thiodiglycol and water. No overt signs of toxicity attributed to HD were manifested in the animals. The rats did not exhibit any other signs of either irritation or overt toxicity during either the exposure or post-exposure periods. No deaths occurred within the 14-day post-exposure period. The product solution from the hot water hydrolysis (neutralization) of HD does not appear to pose an acute inhalation hazard.

14. SUBJECT TERMS

Acute inhalation exposure	HD	Toxicology	Rats
Hot water hydrolysis	DOT	Thiodiglycol	

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PREFACE

The work described in this report was authorized under Sales Order No. 7J1P3A. This work was started in January 1997 and completed in April 1997. The experimental data are recorded in laboratory notebook 97-0016. The storage location for all the raw data and final report are in the Toxicology Archives, Building E-3150.

In conducting the research described in this report, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals, "National Institute of Health Publication No. 85-23, 1996, as promulgated by the Committee on Revision of the Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources, National Research Council, Washington, DC. These investigations were also performed in accordance with the requirements of AR 70-18, "Laboratory Animals, Procurement, Transportation, Use, Care, and Public Affairs," and the Laboratory Animal Use and Review Committee (LAURC), U.S. Army Edgewood Research, Development and Engineering Center (ERDEC),* which approves all ERDEC research protocols requiring laboratory animals. This project, assigned LAURC Protocol No. 97-318, was approved on 6 March 1997.

The performance of this study was consistent with the objectives and standards in "Good Laboratory Practices for Nonclinical Laboratory Studies" (21 CFR 58, Food and Drug Administration, U.S. Department of Health and Human Services, April 1988).

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Acknowledgments

The authors would like to thank the Veterinary Support Team, Renee Crowley and Joseph Hill, Life Sciences Department, ERDEC, and John Rickerl and Jacqueline Scotto, Geo-Centers, Inc., for their help in caring for and handling the animals in this study.

*Now known as the U.S. Army Edgewood Chemical Biological Center (ECBC).

QUALITY ASSURANCE

This study, conducted as described in Protocol 97-318, was examined for compliance with Good Laboratory Practices as published by the U. S. Environmental Protection Agency in 40 CFR Part 792 (effective 17 Aug 1989). The dates of all inspections and the dates the results of those inspections were reported to the Study Director and management were as follows:

Phase Inspected	Date	Date Reported
Study parameters and exposure	03 Apr 97	03 Apr 97
Data and Final Report	03 Feb 99	03 Feb 99

To the best of my knowledge, the methods described were the methods followed during the study. The report was determined to be an accurate reflection of the raw data obtained.

Dennis W. Johnson 3 FEB 99

DENNIS W. JOHNSON
Quality Assurance Coordinator
Research and Technology Directorate

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TABLES

ACUTE INHALATION TOXICITY OF CHEMICALLY NEUTRALIZED HD IN RATS

1. INTRODUCTION

Sulfur mustard ["mustard gas," bis(2-chloroethyl) sulfide, CAS # 505-60-2, military designation: HD] is a potent vesicant and biological alkylating agent.[1] It was first used by the German Army in WWI and has since been stockpiled for use as a chemical warfare agent (CWA) by a number of countries, including the United States.

Public Law 99-145 and subsequent amendments direct the U.S. Army to dispose of the U.S. stockpile of unitary chemical weapons. Incineration is the current baseline technology to accomplish this task. However, public concern about potential risks to human health and safety from agent incineration has prompted the exploration of alternate technologies. The Alternative Technology Program (ATP) was established to develop a process other than incineration for the disposal of CWAs, sulfur mustard (HD) and VX.

Over the past 2 years, a Research and Development (R&D) program demonstrated that HD could be rapidly hydrolyzed (destroyed) in hot water. Hydrolysis dechlorinates HD, providing a significant detoxification.[2] The resultant product solution (effluent) consisting primarily of thiodigylcol (TDG) and water would allow the solution to be transported as a hazardous waste. Since TDG is biodegradable,[3] the product solution could possibly be transported as a nonhazardous waste to a commercial wastewater treatment facility for final treatment.

The transportation of chemicals and/or wastestreams from chemical processes must meet Department of Transportation (DOT) guidelines to ensure proper packaging and handling of material. Guidelines for the safe transportation of chemicals are based on information from toxicity tests conducted in accordance with the Code of Federal Regulation (CFR) 49, Part 173.132 (10/1/94 Edition).[4] Currently, there is a lack of toxicity information on neutralized chemical agent wastestreams. An inhalation toxicity study was performed to help determine what DOT toxicity packing group was appropriate for the neutralized mustard (HD).

2. MATERIALS AND METHODS

2.1 Chemicals.

Agent.

Munitions grade HD (TC Serial # 094102, Sample ID No. HD-S-6176-CTF-N) stored in ton containers at Aberdeen Proving Ground consisted of 92.9 $\pm$ 0.1 wt%* HD as determined by Gas Chromatography/Thermoconductivity Detection and 91.4% HD as determined by GC/Mass Spectrometry.

*Mean of 3 injections.

2.2 Process Chemistry (Chemical Neutralization Technology).

The process chemistry was performed at the bench scale level in a 12 L Mettler RC1 Reaction Calorimeter [Building E3510, U.S. Army Edgewood Research, Development and Engineering Center (ERDEC)].* The reaction consisted of mixing field grade HD (3.8%) with tap water (96.2%) at 90 °C to produce the hydrolysate (Equation 1).

2.2.1 Hydrolysis.

$$\underset{\text{(HD)}}{Cl\,CH_2CH_2SCH_2CH_2Cl} + 2\,H_2O \xrightarrow[90\,^\circ C]{} \underset{\text{(TDG)}}{HOCH_2CH_2SCH_2CH_2OH} + 2\,HCl \qquad (1)$$

2.2.2 Neutralization.

$$2\,HCl + 2\,NAOH \longrightarrow 2\,NaCl + 2\,H_2O \qquad (2)$$

The resulting hydrolysate was a liquid that consisted primarily of water (97%) and TDG (2.4%). Following completion of the reaction, the solution was adjusted to pH 12 using 18% NaOH solution (Equation 2). The amount of unreacted HD present in the sample measured by Hewlett-Packard 5972 GC/Mass Selection Detector [(MSD) scan mode] was <4 ppb. The U.S. Army drinking water limit for HD is 200 ppb.[5]

Samples from the process stream were collected for either chemical characterization of the neutralized HD or toxicity testing. Stream sample no. M12-D1-102-HW-0427 was used for the inhalation exposure as well as in concurrent dermal toxicity studies.[6]

2.3 Animals.

Young adult, male (106 - 126 g) and female (115 – 137 g) Sprague-Dawley rats were obtained from Charles River Laboratories, Incorporated (Wilmington, MA). The animals were quarantined and evaluated for general condition and health status. The animals were then identified by permanent marker (tail) and housed in plastic rat cages in the animal holding facility (E3222). Housing conditions were 12-hr light/dark cycle with 22 ± 4 °C temperature and 40-70% relative humidity (RH). Certified commercial rodent ration (PMI Feeds, Incorporated, St. Louis, MO) and water were available *ad libitum*, except during testing.

Prior to testing, all animals were weighed, numbered and randomly placed into groups. Animal weight ranges on the day of exposure were 199 - 238 g (five males) and 169 - 191 g (five females).

No controls were required for DOT toxicity testing. However, one male and one female rat outside of the exposure group were submitted for serological health monitoring and necropsy at the beginning and end of the 14-day study period.

*Now known as the U.S. Army Edgewood Chemical Biological Center (ECBC).

2.4 Toxicity Testing.

2.4.1 Inhalation Exposure System.

Animal exposures were conducted in a 250-L dynamic airflow inhalation chamber. The chamber flow rate, and test compound feed-rate were determined during the calibration period to achieve the exposure concentration of 5 mg/L. To determine chamber concentration, aerosols were collected onto filter pads and subsequently analyzed. Particle size was determined using a cascade impactor.

The aerosol generation system, located on top of the 250-L chamber, consisted of a 1-L glass reservoir, which contained the test solution, a fluid metering pump (Fluid Metering, Incorporated, Oyster Bay, NY), and an atomizer. Aerosol generation was achieved as the liquid pump drew the test solution from the reservoir and dispensed it at a set rate (2.8 mL/min) into the atomizer. A volume of compressed air (25 psi) was directed through the atomizer, which in turn circulated the aerosol into a glass mixing bowl and into the chamber inlet. The chamber flow rate was then adjusted to 530 L/min to achieve the desired aerosol concentration. Chamber parameters monitored during exposure included temperature and RH. Chamber flow rate was measured with a thermo-anemometer (Model 8565, Alnor, Skokie, IL) before and after exposure.

2.4.2 Acute Inhalation Exposure.

The acute inhalation exposure was set up according to DOT guidelines described in CFR 49, Part 173.132 - 173.133 (10/1/94 Edition).[4] These guidelines determine the packing group for poisonous materials (Class 6, Division 6.1) based on the toxicity (mortality) observed from animal exposure to various aerosol concentrations (Table 1). Sprague-Dawley rats (five male and five female) were exposed (whole body) to aerosols from the HD/H_2O process stream for 1 hr at the highest packing group (>2, ≤ 10 mg/L) and observed for 50% lethality within a 14-day post-exposure period. Control animal exposures were not required for DOT testing.

Table 1. DOT Hazard Classification and Packaging Categories for Division 6.1 Mixtures*

Inhalation Toxicity Testing	
Packing Group	Inhalation toxicity by dusts and mists LC_{50} (mg/L)
I	≤ 0.5
II	> 0.5, ≤ 2
III	> 2, ≤ 10

*The mixture is classified as a 6.1 inhalation poison if lethality occurs in half of the animals tested within 14 days post-exposure.

2.4.3 Sample Collection and Analysis.

Aerosol concentrations of neutralized HD in the chamber were determined by drawing air through glass fiber filter pads (2 L/min) at the animals breathing zone and quantitating for TDG. Samples were collected at 15-min intervals starting 5 min into exposure. Collected filter pads were desorbed with acetonitrile, and the TDG in the solution was then derivitized with N,O-bis[trimethylsilyl]trifluoroacetamide (BSTFA) + 1% trimethylchlorosilane (TMCS*). The derivitization procedure consisted of adding a 50:50 mixture of sample/standard plus derivative to a 1 mL crimp top vial (Pierce Chemical Company, Rockford, IL), which was then heated at 100 °C for 30 min. Derivitized samples and standards were subsequently analyzed by GC with flame photometric detection [(FPD) sulfur mode]. Samples were quantitated by comparing the sample peak areas to a calibration curve (linear regression, $R^2 > 0.99$) established from daily injections of TDG standards. Standards were prepared from serial dilutions of TDG in acetonitrile. Thiodiglycol (99%+) was obtained from Aldrich Chemical Company, Incorporated (Milwaukee, WI). The GC parameters for quantitation of TDG are listed in Table 2.

Table 2. GC Parameters for Quantitation of Thiodiglycol in the Chamber

Gas chromatograph	Hewlett Packard 5890
Capillary column	DB-5, 30 m x 0.53 mm i.d., x 1.5 µm film thickness
Injection volume	1 µl (autosyringe)
Column flow (He)	3.5 mL/min (head pressure = 2.0 psi)
Septum purge	3 mL/min
Detector flow	400 mL/min (air); 29 mL/min (hydrogen)
Detector temperature (FPD)	225 °C
Injector temperature	180 °C
Injection mode (splitless)	Single gooseneck (4 mm), Restek, Bellefonte, PA
Purge	Off Time: 0.00 min; On Time: 0.50 min
Col temperature program	60 °C (hold 1 min) to 225 °C @ 25°/min (hold 10 min)

The aerodynamic particle size was measured using a 10-stage cascade impactor (model 2210-K, Graseby-Andersen, Atlanta, GA). Chamber air samples were drawn through the impactor at 7 L/min during the midpoint of the exposure. Aerosols drawn through the impactor were collected onto glass fiber substrates beneath each stage. The substrates were subsequently weighed to determine mass collected at each size range. The mass median aerodynamic diameter (MMAD) and geometric standard deviation (σg) were determined by log-normal regression (least squares method) of particle size versus cumulative relative mass.

*N,O-bis[Trimethylsilyl] trifluoroacetamide + Trimethylchlorosilane

3. RESULTS

3.1 Neutralized HD Test Solution.

Chemical analysis on the neutralized HD/H_2O process stream was conducted by the Analytical Chemistry Team, ERDEC.* The amount of HD in the final reaction solution was below the detectable limit (<4 ppb) via GC/MSD. Thiodiglycol was the main reaction product from the hydrolysis of HD in water. The test solution contained 2.36% (23,600 mg/L) TDG as determined by high performance liquid chromatography (HPLC) via diode array detection. A summary of the components present during the initial and final reaction stages is provided in Appendix A. Solvent (chloroform) extractable organics present in the hydrolysate and identified by GC/flame ionization detection (FID) (retention time) and GC/MS/Chemical Ionization (CI) are listed in Appendix B.

3.2 Chamber Parameters.

The chamber was operated under slight negative pressure (0.07 in. water, magnehelix). Chamber flow (534 L/min) was measured with a thermo-anemometer (Alnor, Niles, IL) immediately before and 15 min after exposure. The calculated chamber equilibration time (t_{99}) was 2.2 min.

3.3 Aerosol Concentration During Exposure.

The aerosol chamber atmosphere consisted primarily of water and TDG. The mean TDG concentration during the 1-hr exposure was 125 μg/L ± 14 (Table 3). Calculations for the nominal aerosol concentration (5.4 mg/L of H_2O/TDG) and percent recovery of TDG are shown in Table 4.

Table 3. Thiodiglycol Concentration and Particle Size During 1-Hour Exposure to Neutralized HD

	Sample Time (min)	TDG (μg/L)	Particle Size MMAD (μ)	σg
	5 - 7	130		
	20 - 22	129		
	30 - 38		3.27	3.31
	35 - 37	121		
	50 - 52	118		
Mean	125 μg/L ± 14			

*Now known as ECBC.

Table 4. Nominal Aerosol Concentration and Percent Recovery of TDG in the Exposure Chamber

Total Aerosol Concentration (mg/L) (Nominal)	$= \frac{2.8 \text{ mL/min} \times 1.0223 \text{ g/mL (density)} \times 10^3 \text{ mg/g}}{534 \text{ L/min}}$ = 5.4 mg/L
TDG in Chamber (μg/L) (Nominal)	= 5.4 mg/L (total aerosol) x 2.4% TDG x 10^3 μg/mg = 130 μg/L
% Recovery $\frac{125 \text{ μg/L TDG (analytical)}}{130 \text{ μg/L TDG (nominal)}} \times 100$	= 96%

3.4 Aerosol Particle Size.

The MMAD was 3.27 μg and the σg was 3.31, indicating a polydispersed aerosol (Table 3).

3.5 Toxicology.

Animals were monitored for toxic signs and behavioral changes during exposure to the aerosols from the HD/H_2O neutralization process. They showed no toxic signs during either the exposure or post-exposure periods. Particular attention was given to the eye since it is the most sensitive indicator to mustard exposure. There was no sign of lacrimation from the eyes or swelling and edema around the conjunctiva and eyelids. There were no latent HD-related effects during the post-exposure period. All animals showed a normal increase in weight, and there were no deaths at the 14-day post-exposure period.

4. DISCUSSION

The HD was chemically neutralized by hot water (90 °C) hydrolysis to produce a product solution of low toxicity (no overt signs of toxicity due to agent, no mortalities). Low acute toxicity of the neutralized HD solution would permit handling in a manner similar to industrial and/or nonhazardous wastes that are commercially transported. An acute inhalation toxicity study was conducted to determine if an HD neutralized process stream would pose an inhalation hazard (DOT Class 6 poison) should either a leak or a spill occur during its transport. Due to the low vapor pressure of the material, either spillage or leakage would most likely pose an aerosol inhalation hazard as opposed to a vapor one. Therefore, DOT guidelines for an aerosol exposure were followed.

DOT inhalation tests were used to determine the hazard classification of a material, and are based on a rat inhalation exposure paradigm (test article concentration ≤ 0.5 - ≤ 10 mg/L; exposure duration 1 hr). In this study, rats were exposed to the highest aerosol exposure level (Packing Group III, Exposure range $> 2, \leq 10$ mg/L) to determine if an LC_{50} would occur within a 14-day post-exposure period. Rats were exposed to an aerosol concentration of 5.4 mg/L. The exposure atmosphere consisted primarily of TDG (125 μg/L) and water.

The primary reaction product, TDG (CAS # 111-48-8) is an aliphatic mercaptan. Acute inhalation may cause irritation to the eyes, mucous membranes, and upper respiratory tract. In this study, the rats did not exhibit any irritation or overt toxic effects from the 1-hr exposure at 125 μg/L. No other inhalation toxicity studies for TDG have been reported in the literature.

Concurrent studies on the dermal effects of the test material on rabbits exposed to a 1000-mg/kg dose for 4 hr resulted in neither toxic signs nor dermal irritation and no lethal effects at 14-days post-exposure.[6] These results indicate that the dermal toxicity of the neutralized HD was less toxic than Packing Group III materials according to biological criteria set forth in 49 CFR (DOT).[4]

The toxicological characterization of the product solution from the HD/H_2O reaction as assessed via inhalation exposure showed no animal mortality and no observable animal irritation or toxic signs (immediate or latent) at the highest packing group level ($>2, \leq 10$ mg/L). Based on these findings, the product solution appears to be less toxic than a Class 6 poison (Packing Group III) material as set forth in 49 CFR (DOT).[4]

5. CONCLUSIONS

Based on the findings of this study, the following conclusions can be made:

The aerosol inhalation toxicity of the reation product from neutralized HD was less toxic than "Packing Group III materials" according to biological criteria set forth in Department of Transportation CFR 49 (Part 173.132 - 173.133, Class 6, Division 6.1, pp 504-508, October 1, 1994).

The product solution (thiodiglycol/water) does not appear to pose an acute inhalation hazard.

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APPENDIX A

COMPOSITION OF HD/H_2O TEST SOLUTION

I. Initial Reaction Mass

A. Reactants:

HD	0.38 kg (92.9 % Purity)	3.8 %
Water	9.7 kg	96.2 %

B. Impurities Present in HD:

Q (approximately by GC-TCD)	5.5 %
1,2 dichloroethane (approximately by GC-TCD)	0.35 %

C. Metals: Present from Ton Container

Arsenic	19 ppm
Copper	92 ppm
Iron	5,035 ppm
Sulfur	264,420 ppm

II. Final Reaction Mass (Neutralized HD/H_2O Solution)

A. Solvents/Reactants:

Water:	Solvent
2,2'-dichlorodiethyl sulfide (HD)	< 4 ppb
Thiodiglycol	Reactant (2.4%)

B. Additions:

NaOH	Adjust pH to 12.2

C. Metals: Present from Stainless Steel Reactors

Chromium	103 ppm

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APPENDIX B

SUMMARY OF COMPOUNDS IDENTIFIED
BY GC/MS/CI AND GC/FID IN THE CHLOROFORM EXTRACTION
OF SAMPLE M12-D1-103-HW-0427

ANALYTICAL REQUEST AND RESULTS

TO: (REQUESTOR, PLEASE FILL IN NAME AND ADDRESS)	DATE / PHONE NO.
R. S. Lindsay, Design Evaluation Lab SCBRD-ENM-S E3510	DATE 2/11/97 PHONE NO. X2801

FROM: Analytical Chemistry Team

ANALYSIS OF (structure or further description IF UNCLASSIFIED on reverse side)

HD/Water Reaction Products

SAMPLE NO.

M12-D1-103-UW-0427

TOXIC   NON-TOXIC   MOL. WT.   EMP. FORM

Toxicity Undetermined

DETERMINE

Organics

RESULTS AS FOLLOWS BY: J. Janes/21Feb97
D. Rohrbaugh/13Feb97

Extraction.

Twenty-five milliliters of the aqueous sample was filtered, pH adjusted and extracted with two milliliters of chloroform. The extracted sample was submitted for analyses.

Analysis.

See attached sheets for organic characterization.

Organic composition using GC/FID consisted of the following compounds:

Compound	Retention Time	Conc. (in extract)	Conc. (in aqueous)
1,4-Thioxane	4.218min	0.236mg/mL	37.76ppm
1,4-Dithiane	7.780min	1.769mg/mL	283.04ppm
TDG	9.746min	0.036mg/mL	5.76ppm
HO-CH_2CH_2-S-CH_2CH_2-S-CH_2CH_2-OH	16.895min	0.038mg/mL	6.08ppm
HO-CH_2CH_2-S-CH_2CH_2-O-CH_2CH_2-S-CH_2CH_2-OH	20.092min	0.023mg/mL	3.68ppm
HO-CH_2CH_2-S-CH_2CH_2-S-CH_2CH_2-S-CH_2CH_2-OH	22.593min	0.074mg/mL	11.84ppm

TEAM LEADER	DATE
Michael W. Ellzy	3 Mar 1997

SCBRD Form 49, 15 Jun 94 replaces SMCCR Form 50, 1 May 85 which is obsolete.

APPENDIX B

GC/MS/CI Characterization of M12-D1-103-HW-0427 $CHCl_3$ Extract

Scan No	RT (min)	MW	Compound	Area %
256	3:01	104	1,4-Thioxane	6.87
280	3:18	104,106	CH_2=$CHSCH_2CH_2OH$, $EtSCH_2CH_2OH$	2.36
320	3:46	182	$Cl_2CHOCHCl_2$	0.17
327	3:51	142	$ClCH_2CH_2OCH_2CH_2Cl$	0.16
332	3:55	132	Unknown	0.48
357	4:12	132	Unknown	0.22
367	4:19	132	Unknown	0.26
411	4:50	120	1,4-Dithiane	74.3
424	5:00	118	Unknown	0.07
446	5:15	134	Unknown	0.18
481	5:28	156	$ClCH$=$CHSCH_2CH_2Cl$ or Isomer	0.72
503	5:55	122	Thiodiglycol	8.04
523	6:09	148	Unknown	0.26
541	6:22	148	Unknown	0.28
579	6:49	187	$RSCH$=CH_2	0.29
582	6:51	189	RSEt	0.51
605	7:07	132	Unknown	0.34
710	8:21	164	CH_2=$CHSCH_2CH_2SCH_2CH_2OH$	2.42
727	8:33		Unknown	0.16
731	8:36	162	Unknown	0.17

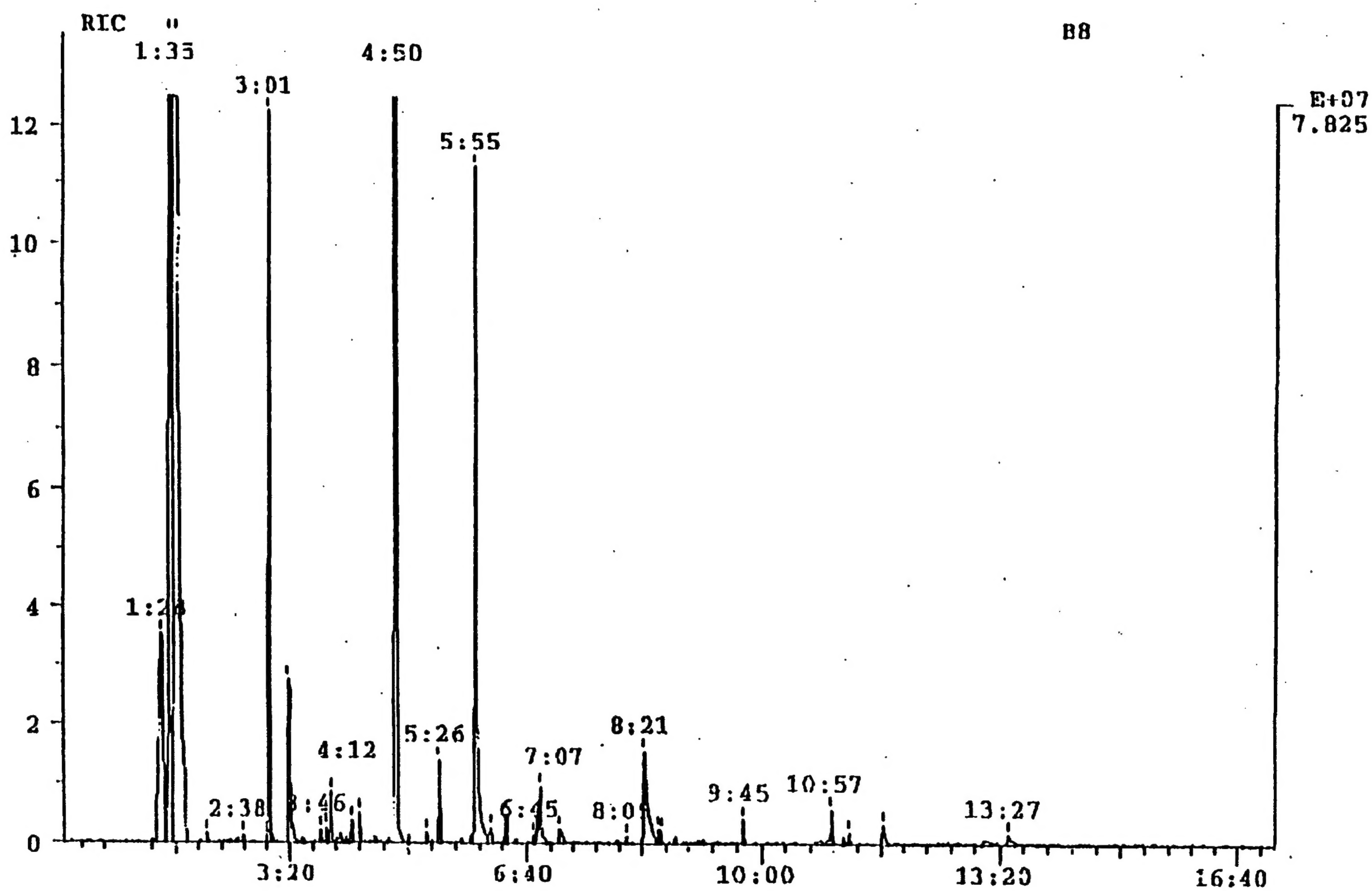
829	9:45	222	Diethylphthalate	0.34
931	10:57	206	DCCDI	0.37
946	11:07	247	$RSCH_2CH_2SCH{=}CH_2$	0.09
953	11:12	208	$CH_2{=}CHSCH_2CH_2OCH_2CH_2SCH_2CH_2OH$	0.17
994	11:41	288	RSR	0.40
1145	13:27	224	DCCDI Urea	0.38

R = 2-diisopropylaminoethyl
DCCDI = Dicyclyclohexylcarbodiimide

Run obtained 13 Feb 97 on TSQ-7000 (File m12ci)

Note: Presence of VX degradation products and DCCDI suggests reactor may have been used for VX prior to this run without complete cleaning.

CHRO: m12ci 23-FEB-97 Elapse: 13:41.0 994
Samp: ML2-D1-103-HW-C427 CHCl3 Extract Start : 16:26:02 1832
Comm: GC/MS/CI 30m DB-5 60-270C at 15C/min
Mode: CI +Q1MS LMR UP LR
Oper: DKR Inlet :



APPENDIX B

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APPENDIX C

REPORTED DEVIATIONS FROM THE ANIMAL USE PROTOCOL

In accordance with ISO Guide 25, the following deviations from the animal use protocol are reported:

(1) The weight of one exposed female rat (169 g) was slightly below the exposure weight range of 175 - 250 g.

(2) Ambient housing conditions for the animals are to be maintained at 70 ± 5 °F and 40 - 70% relative humidity (RH) throughout the animal housing period. Deviations of temperature (75 - 80 °F) and RH (28 - 39%) occurred at various times during this period. However, the veterinarian does not believe this caused any deleterious effects on the animals.

These deviations do not appear to have any deleterious effect on either the study or interpretation of the data.